DIAGNOSTIC AGENT AND METHOD FOR DETECTION OF CANCER AND A MEANS FOR TREATMENT OF SAME

Field of the Invention

This invention relates to a diagnostic agent and a method for detection of cancer, in particular a method for detection of colorectal cancer (cancer of the large intestine) in a tissue biopsy of the human colon or rectum and a means for treatment of carcinoma, in particular colorectal carcinoma.

Background of the Invention

Cancer is known to have become increasingly important in all countries. Treatment of cancer and therapeutic success depend to a significant extent on prompt detection of the cancer. Therefore, there is a great demand for reliable cancer diagnostic agents, in particular those that allow detection of metastases and micrometastases, even when there are no definite histological findings or when the histological findings are negative.

Colorectal cancer is the second main cause of cancer fatalities; the incidence has been increasing steadily and it often recurs after a curative surgical operation. Colorectal cancer (malignant tumors of the colon and rectum) are occurring in a constantly increasing incidence in industrial countries and constitute the second most common type of cancer in men and the third most common in women. Colorectal cancer constitutes 50% of malignancies. There are more than 200,000 new cases of colorectal cancer each year, and more than 100,000 patients die of it. Colorectal cancer is thus the second leading cause of death due to cancer.

Colorectal cancer may develop *de novo* or as part of an adenoma-carcinoma sequence in an adenomatous polyp. The probability of a malignancy is between 1% and 40% in the case of adenomas. To this extent, the patients with colorectal polyps constitute a risk group.

For this reason, early detection of colorectal cancer in adenomas and reliable differentiation from benign colorectal tissue are of crucial importance, especially for the prognosis and course of treatment.

The diagnosis and prognosis for this type of cancer are influenced by a variety of properties which are present at the time of the initial diagnosis. These factors include age, sex, duration of symptoms, condition of the intestinal obstruction, tumor localization, the need for a blood transfusion and the quality of the surgical intervention. Although a number of tumor properties such as vascular lymphatic invasiveness, the degree of differentiation and the preoperative titer of conventional tumor markers have shown a prognostic relevance, but there are no suitable markers for detection of early stages of cancer (benign colorectal precursors (adenomas) that become malignant) or for histopathologically unremarkable micrometastases (minimal residual disease) which may be responsible for a recurrence of the carcinoma, even after curative surgical resection. The tumor markers CEA, CK 19 and CK 20 which have been used in the past are indicative of the current prognosis but are unreliable in a differential diagnosis.

Object of the Invention

Therefore, the object of this invention is to make available a reliable diagnostic agent and a method for detection of colorectal cancer and also to permit the use of an effective agent for treatment of colorectal cancer.

Summary of the Invention

To achieve this object, this invention proposes that a tissue biopsy of the human colon or rectum should be tested for the presence of HERG potassium channels.

Therefore, the object of this invention is a diagnostic agent for detection of colorectal cancer with which it is possible to detect the presence of at least one HERG potassium channel in a tissue specimen of the human colon or rectum, which is free of HERG potassium channels in a healthy person, or in body fluids. Another object of the present invention is a method for diagnosing colorectal cancer, whereby the presence of at least one HERG potassium channel is detected in a tissue biopsy of the human colon or rectum, which is free of HERG potassium channels in a healthy person, or in lymph nodes or in a body fluid.

Germany). The amount and purity of the RNA were determined by spectrophotometry at wavelengths of 260 nm and 280 nm.

The Colo-205 colorectal carcinoma cell line was used as a positive control for detecting the sensitivity of these experiments.

Reverse transcription

The cDNA was synthesized from 2 µg total RNA in a volume of 20 µL reaction mixture containing 4 µL of 5× reaction buffer (50 mmol/L Tris-HCl, pH 8.3, 75 mmol/L KCl and 3 mmol/L $MgCl_2$), 500 µmol/L dNTP, 100 µmol/L solution of poly-dT15 primer (Roche Diagnostic, Mannheim, Germany) and 500 units of Superscript II (Gibco BRL, Gaithersburg, MD, USA). The mixture was incubated for 60 minutes at 42°C, then heated for two minutes at 90°C and next cooled on ice.

Primer sequences for the RT-PCR analysis:

The following primer sequences were used for the subsequent RT-PCR tests:

The primer sequences for CEA mRNA were:
A. 5'-TCTGGAACTTCTCCTGGTTCTCTCAGCTGG-3' (SEQ ID NO:1) for the outer sense;
B. 5'-TGTAGCTGTTGCAAATGCTTTAAGGAAGAA-3' (SEQ ID NO:2) for the antisense; and
C. 5'-GGGCCACTGTCGGCATCATGATTGG-3' (SEQ ID NO:3) for the inner sense cases.

The primer sequences for CK-19 mRNA were:
A. 5'-GTGGAGGTGGATTCCGCTCC-3' (SEQ ID NO:4) for the outer sense;
B. 5'-TGGCAATCTCCTGCTCCAG-3' (SEQ ID NO:5) for the outer antisense;
C. 5'-ATGGCCGAGCAGAACCGGAA-3' (SEQ ID NO:6) for the inner sense; and
D. 5'-CCATGAGCCGCTGGTACTCC-3' (SEQ ID NO:7) for the inner antisense cases.

The primer sequences for CK-20 mRNA were:
A. 5'-GCGTTTATGGGGGTGCTGGAG-3' (SEQ ID NO:8) for the outer sense;

B. 5'-AAGGCTCTGGGAGGTGCGTCTC-3' (SEQ ID NO: 9) for the outer antisense;
C. 5'-CGGCGGGGACCTGTTTGT-3' (SEQ ID NO: 10) for the inner sense; and
D. 5'-CAGTGTTGCCCAGATGCTTGTG-3' (SEQ ID NO: 11) for the inner antisense cases.

The primer sequences for the HERG mRNA were:
A. primer up 5'-AGCTGATCGGGCTGCTGAAGACTG -3' (SEQ ID NO: 12) and
B. primer down 5'-AATGAGCATGACGCAGATGGAGAAG-3' (SEQ ID NO: 13).

To investigate the integrity of the extracted RNA and to ensure that equimolar RNA was used, the extracted RNA was tested with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) by RT-PCR. The primary sequences for GAPDH were:
5'-CCACCCATGGCAAATTCCATGGCA-3' (SEQ ID NO: 14) sense and
5'-TCTAGACGGCAGGTCAGGTCCACC-3' (SEQ ID NO: 15) antisense primers.

Reverse transcriptase polymerase chain reaction (RT-PCR):

Two-step RT-PCR was used for amplification of cDNA of CEA, CK-19 and CK-20 as well as HERG.

PCR was performed as follows: PCR was performed in a volume of 50 µL and 2 µg of the total RNA per sample. For the first PCR round, 2 µL aliquots of the cDNA solution were mixed with 10.5 µL of the PCR reaction mixture containing 1.25 µL 10 × PCR buffer (10 mmol/L Tris-HCl, pH 8.3, 50 mmol/L KCl and 1.5 mmol/L $MgCl_2$), 200 µmol/L dNTP, 0.5 µmol/L of each primer and 2.5 units of platinum Taq polymerase (Gibco BRL, Gaithersburg, MD).

The reaction was continued in a PCR Thermocycler (Biometra, Göttingen, Germany). For CEA, CK-19 and CK-20 amplification, the following conditions were used: activation of Taq polymerase for four minutes at 95°C, template denaturing for 45 seconds at 95°C, annealing for 45 seconds at 60°C and elongation for 45 seconds at 72°C for 20 cycles. HERG amplification was performed for four minutes at 95°C, for one minute at 55°C, for one minute at 72°C and for one